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Award Number: W81XWH-05-1-0056

TITLE: Cancer Localization in the Prostate with F-18 Fluorocholine Positron Emission Tomography

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REPORT DATE: January 2008

TYPE OF REPORT: Addendum to Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| **1. REPORT DATE** *(DD-MM-YYYY)* | **2. REPORT TYPE** | **3. DATES COVERED** *(From - To)* |
|---|---|---|
| 01-01-2008 | Addendum to Final | 20 DEC 2006 - 19 DEC 2007 |

**4. TITLE AND SUBTITLE**

Cancer Localization in the Prostate with F-18 Fluorocholine Positron Emission Tomography

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**
W81XWH-05-1-0056

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**
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**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

The Queen's Medical Center
Honolulu, HI 96813-2413

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSOR/MONITOR'S ACRONYM(S)**

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**
The project evaluated fluorine-18 (F-18) fluorocholine positron emission tomography (PET) as an imaging technique for delineating malignancy in the prostate gland. The technique measures tissue metabolism of fluorocholine, a substrate that is preferentially metabolized by cancer cells due to malignant over-expression of the choline transporter and choline kinase enzyme. Based on this measurement, it was proposed that cancerous tissue can be differentiated from benign tissue in the prostate. Project Scope: Men with prostate cancer undergoing treatment with radical prostatectomy surgery were recruited for pre-operative PET scanning to measure fluorocholine uptake in the prostate gland. Imaging results were compared to histopathologic analyses of the prostatectomy specimen to determine the accuracy of prostate cancer sextant localization on the basis of measured fluorocholine uptake. The data acquired thus far with conventional PET in 15 subjects (2 pilot subjects and 13 recruited by the study) was summarized in the 2006 final report. Since then, the project scope has been modified to incorporate the use of PET/CT and other new PET imaging technologies. A no-cost extension is being requested to continue the project using PET/CT with the goal of accruing at least ten additional subjects. Additional immunohistochemical analyses are planned once additional prostate specimens have been collected. To better facilitate this task, a method to establish spatial correspondence between histology slides and ex vivo MRI was developed this year to allow histological correspondence to be propagated to the in vivo imaging space. This latter project outcome should aid histopathologic correlations in future validation studies of fluorocholine PET and other prostate imaging techniques.

**15. SUBJECT TERMS**
Prostate Cancer, Localization, Positron Emission Tomography, Fluorocholine

| **16. SECURITY CLASSIFICATION OF:** | | | **17. LIMITATION OF ABSTRACT** | **18. NUMBER OF PAGES** | **19a. NAME OF RESPONSIBLE PERSON** |
|---|---|---|---|---|---|
| **a. REPORT** | **b. ABSTRACT** | **c. THIS PAGE** | | | **19b. TELEPHONE NUMBER** *(include area code)* |
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## INTRODUCTION

The objective of this project is to develop and evaluate fluorine-18 labeled fluorocholine as an imaging agent for positron emission tomography (PET) detection of malignancy in anatomical sextants of the prostate gland. The rationale for evaluating fluorocholine as an oncologic tracer applicable to prostate cancer is based on observations of increased choline and fluorocholine metabolism in malignant prostate tissue relative to normal tissue. Information obtained from fluorocholine PET regarding tumor location and volume has potential value in guiding transrectal biopsy or in refining therapeutic approaches against organ-confined prostate cancer.

This project, with a planned enrollment of 25 subjects with prostate cancer who have elected radical prostatectomy, will study investigational pre-operative fluorocholine PET scanning of the prostate using correlation with step-section prostate histopathology to assess the accuracy of sextant detection of prostate malignancy based on this technique. With the commercial introduction of PET/ X-ray computerized tomography (PET/CT), the original project was updated to replace stand-alone PET imaging with PET/CT. Improved anatomical localization by PET/CT is expected to improve the accuracy of localizing fluorohcoline uptake in prostate gland sextants.

This addendum to last year's final report covers work performed during the period from 20 December 2006 to 19 December 2007. During this period, PET/CT devices were installed at both The Queen's Medical Center and Tripler Army Medical center. IRB-approved study protocols were revised this year to incorporate corresponding changes in protocol. The FDA IND for fluorocholine was updated to include Tripler Army Medical Center as a site of radiopharmaceutical fluorocholine administration to human subjects. During this period, a method was also developed to facilitate spatial correspondence between prostate histopathologic data and tomographic imaging data. This addendum summarizes these research activities.

## BACKGROUND

The declining rate of prostate cancer deaths in the United States has been attributed to improved rates of cancer screening. However, prostate cancer is still the most prevalent cancer affecting American men. This disease is expected to account for 29% of the new male cancer cases in 2007 while remaining the second leading cause of male cancer death with an estimated 27,050 deaths this year *(1)*. The low ratio of deaths to incidence underscores the fact that treatment for early and localized prostate cancer is potentially curative. If treated at an organ-confined stage, the expected 5-year survival from prostate cancer is 100% as compared to a 33% 5-year survival for metastatic prostate cancer. *(1)*. In vivo imaging capable of detecting prostate cancer can contribute to increasing the number of patients receiving appropriate therapy by way of earlier detection and pre-treatment localization of disease.

## BODY

### STUDY REVISION (related to SOW Task 1)

For this project, PET/CT effectively replaces the use of stand-alone CT and magnetic resonance imaging (MRI) of the pelvis. The PET/CT devices became available for clinical use in April 2007 and September 2007 at Tripler Army Medical Center and The Queen's Medical Center, respectively. Protocols for PET/CT in the research study were finalized shortly thereafter. In addition to utilizing PET/CT, the study protocol was revised to allow Tripler to be an imaging site for the study. The Chief of Nuclear Medicine at Tripler, Dr. Douglas Prager, was added as a study investigator authorized to supervise fluorocholine

administrations. The FDA IND application for fluorine-18 fluorocholine was revised to reflect these modifications.

Because the use of PET/CT constituted a significant protocol change, a revised protocol and consent form was submitted for institutional review board (IRB) approval prior to implementation. Radiation dose estimates were updated to reflect the radiation exposure from the low-dose CT to be performed in conjunction with the PET. Following radiation safety and human subjects review, the revised protocols were approved by The Queen's Medical Center Research & Institutional Review Committee (IRB) on 12 July 2007, and by the Tripler Army Medical Center Human Use Committee on 24 September 2007. The revised protocol was subsequently sent to the USAMRMC Human Research Protection Office as part of an annual continuing review report on 2 October 2007. The study is now active with a current protocol expiration date of October 2008. As of December 19, 2007, two subjects have completed participation in the study under the revised protocol. Because new data has not yet been collected from a significant number of subjects, there are no new tabulated results reported in this addendum. Previous research data acquired using stand-alone PET was included in the 2006 Annual Report and has been accepted for publication in a scientific peer-review journal. These results are also included and discussed in Reprint #3 of Appendix 2.

**STUDY METHODS (relevant to SOW Tasks 2 and 3)**

**PET/CT Imaging**

A Discovery LS PET/CT (GE Medical Systems) was installed at Tripler AMC and a Gemini TF PET/CT (Philips Medical Systems) was installed at The Queen's Medical Center. While there are significant technical differences between these two devices, the imaging protocols were standardized to provide similar data sets. The current imaging protocol is briefly described: A sterile intravenous catheter is inserted into an antecubital vein, and the subject is positioned supine on the PET/CT scanning table. A limited low-dose CT scan of the pelvis is performed without intravenous contrast (CT protocol: scout image with kv/mA 120/10, followed by helical rotation CT covering the pelvis with rotation time 0.8s, thickness 3.75 mm, interval 3.27 mm, pitch 1.5:1, kV/mA 140/110 with auto mA range 30-210, full field of view). A dose of 0.09 mCi per kg of fluorine-18 fluorocholine is subsequently administered intravenously over 60 seconds followed by an intravenous saline flush. A dynamic emission PET scan of the pelvis is performed immediately post-injection for up to 20 minutes. Vital signs are recorded before injection and immediately following completion of the PET/CT scan. The entire imaging procedure can be completed in under 30 minutes.

PET emission data is now acquired natively in list-mode. After an interative reconstruction process, the tomographic data is presented in both dynamic and static formats. Data from the Gemini TF system will also be reconstructed using a time-of-flight reconstruction algorithm. Because a whole-body PET/CT scan would have resulted in a higher whole-body radiation dose than a conventional PET (emission and transmission) scan of the body (as employed in the original study protocol), the total radiation dose associated with the amended study protocol was limited by reducing the field of view of the PET/CT. In the current revised protocol, only the prostate and surrounding pelvic lymph node regions are imaged. This and other protocol design considerations are discussed in publication reprint #1 of Appendix 2.

**DATA ANALYSIS (Relevant to SOW Task 3)**

There were no protocol changes related to histopathologic processing of the prostate specimens. During the addendum period, two additional prostate specimens have been sent to the Armed Forces Institute of Pathology for histopathologic analysis. While the clinical pathology has already been completed, digital images of the specimens have not yet been made available for sextant correlation analysis. Three subjects have been recruited since the protocol revision, but only 2 subjects have completed participation. Data from a 3rd subject was not obtained due to temporary malfunction of the PET/CT at Tripler.

**Prostate Specimen Analysis and Development of Ex-vivo Image Registration Technique (Relevant to SOW Tasks 3 and 4)**

A method to automatically register histological slides to ex-vivo MRI was developed during this reporting period. This method results in spatial correspondence between histology slides and *ex vivo* MRI, allowing histological correspondence to be propagated to *in vivo* tomography. The method of registration was implemented in software by Drs. Charles Miller and Hyunjin Park from the image processing laboratory of the University of Michigan Department of Radiology. The implementation utilizes a cost-function based analysis of mutual information with transformations based on thin-plate splines to perform the co-registration. The manual task of correlating histological slides to volumetric images such as MRI is difficult for humans because it is both time-consuming and requires extensive search of a 3D volume for corresponding 2D (ie. slice) results. Computer automation of this task is non-trvial as there are significant differences in image contrast and information content between optical histology and MRI, with significantly higher information content and spatial resolution of the histology slides relative to MRI. The proposed registration method overcomes this challenge by breaking the difficult registration task into easier registration sub-tasks. For this purpose, registrations were performed between the histlogical slides and block face photos, as well as between the block face photos and ex vivo MRI. Results from two registrations tasks are combined to establish registration between histology slides and *ex vivo* MRI. The technical details of this registration approach are included in reprint #4 of Appendix 2.

The ex vivo MRI used for registration was obtained as follows: Upon receipt of the intact prostate specimens fixed in a 10% formalin solution, images were acquired at 7 telsla using a magnetic resonance microscope(MRM) (Bruker Biospec 7T, Bruker Biopsin Corp, Billerica, MA). MRI sequences include rapid acquisition with relaxation enhancement (RARE, field of view (5.8)^3, matrix (256)^3) lasting 9 hours 26 minutes and gradient echo fast imaging lasting 7 hours 16 minutes. Three-dimensional images of the entire prostate specimen and quantitative 2D slice images at selected planes were acquired. After imaging, histologic processing of the prostate specimen was performed by the step-section technique. Completely embedded whole prostate specimens were sectioned at regular 2.2 mm intervals. Block face photos consisting of digital images of the prostate specimen as it is sectioned were also acquired. Thin slices from each section were mounted on large glass slides and stained with hemotoxylin and eosin.

## KEY RESEARCH ACCOMPLISHMENTS

**- A method for registering step-section prostate histology slides and ex-vivo MRI was developed and tested. This method should facilitate more accurate radiopathologic correlations, since the ex-vivo MRI image registered histopathology images are amenable to subsequent co-registrations with in-vivo MRI and other volumetric image data sets.**

**- PET/CT and dynamic imaging were incorporated into the IRB-approved study protocol. The human subjects research protocol is currently active and open to accrual until October 2008.**

## REPORTABLE OUTCOMES

The following reports supplement those listed in the 2006 Project Final Report:

### 2007 PUBLICATIONS:

Kwee SA, DeGrado TR, Talbot JN, Gutman F, Coel MN. Cancer Imaging with Fluorine-18 Labeled Choline Derivatives. Seminars in Nuclear Medicine. 37: 420-428. November 2007.

DeGrado TR, Kwee SA, Coel MN, Coleman RE. The Impact of Urinary Excretion of $^{18}$F-Labeled Choline Analogs. J Nucl Med 2007 48: 1225

Kwee SA, Thibault G, Stack R, Coel M, Furusato B, Sesterhenn I. Use of Step-Section Histopathology to Evaluate 18F-Fluorocholine PET Sextant Localization of Prostate Cancer. Molecular Imaging. 2007 –accepted for publication, in-press.

### 2007 ABSTRACTS, PRESENTATIONS, AND CONFERENCE PAPERS:

Kwee SA, Thibault G, Stack R, Coel M, Furusato B, Sesterhenn I. Non-Invasive Detection and Therapeutic Targeting of Cancer in the Prostate Using Fluorine-18 Fluorocholine Positron Emission Tomography. IMPact (Innovative Minds in Prostate Cancer Today) 2007. Atlanta, GA.

Kwee SA, Thibault G, Stack R, Coel M, Furusato B, Sesterhenn I. Prostate Imaging with 18F-Fluorocholine Using a Whole-Body Positron Emission Tomograph. Nuclear Science Symposium / Medical Imaging Conference - Institute of Electrical and Electronics Engineers 2007. Honolulu, HI.

Park H, Kwee S, Thibault G, Stack G, Furusato B, Sesterhenn I, Meyer CR. Registration Methods for Histological Slides and *ex vivo* MRI of Prostate. Nuclear Science Symposium / Medical Imaging Conference, Institute of Electrical and Electronics Engineers 2007. Honolulu, HI.

**CONCLUSION**

As proposed in the 2006 Final Report, the project scope has been expanded to incorporate PET/CT. Installation of the PET/CT devices was completed this year at the two project performance institutions, allowing dynamic PET data for the project to be acquired in conjunction with anatomical CT. Recruitment for the study has resumed during this period and will continue through a no-cost extension into 2008 with the goal of accruing at least ten additional subjects. Immunohistochemical correlation analysis will be finalized after specimen collections are completed. A method to establish spatial correspondence between histology slides and *ex vivo* MRI was also developed during this period to allow histological correspondence to be propagated to the *in vivo* imaging space. This new registration technique will allow more accurate histopathologic correlation to be performed in future validation studies of fluorocholine PET and potentially other prostate imaging techniques.

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3. Kwee SA, Wei H, Sesterhenn I, Yun D, Coel MN. Localization of primary prostate cancer with dual-phase 18F-fluorocholine PET. *J Nucl Med.* 2006;47(2):262-269.
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## APPENDIX 1: Statement of Work (Revised July 2006)

Cancer Localization in the Prostate with F-18 Fluorocholine Positron Emission Tomography

**Task 1. Study Preparation, Months 1-4:**

a. Finalize research protocol and study-specific forms.

b. Obtain institutional review board (IRB) approval of study protocol and consent form at project sites: Tripler Army Medical Center (TAMC), Queen's Medical Center (QMC), and the Armed Forces Institute of Pathology (AFIP).

c. Orient all study personnel on protocol and methods.

**Task 2. Subject Recruitment and Data Collection, Months 4-20:**

a. Begin subject recruitment at TAMC and QMC. A total of 25 subjects will be recruited from both sites over a 16 month period.

b. Subjects will undergo whole-body F-18 FCH PET or PET/CT scanning to acquire images of the prostate gland.

c. Subjects not undergoing PET/CT will undergo a separate CT at QMC.

d. Following surgery, the prostatectomy specimens will delivered to AFIP for processing and analysis. Analysis procedures include surgical histopathology and immunohistochemical staining for the Ki-67 antigen. The data will be recorded on study-specific pathology forms.

e. All data will be entered into a study database for analysis.

**Task 3. Data Analyses, Months 6 – 20:**

a. PET or PET/CT image analysis will be performed by two physicians.

b. Collected data will be analyzed and correlated in periodic interim analyses. Interim results will be summarized in annual reports.

**Task 4. Final Analyses/Reporting and Design of Secondary Studies, Months 20-24:**

a. Finalize analysis of data and summarize results as stated in the specific aims.

b. Prepare final report and manuscripts for publication.

c. Design secondary studies using the collected data.

## APPENDIX 2:

## REPRINTS ATTACHED ON SUBSEQUENT PAGES:

1. Kwee SA, DeGrado TR, Talbot JN, Gutman F, Coel MN. Cancer Imaging with Fluorine-18 Labeled Choline Derivatives. Seminars in Nuclear Medicine. 37: 420-428. November 2007.

2. Timothy R. DeGrado, Sandi A. Kwee, Marc N. Coel, and R. Edward Coleman The Impact of Urinary Excretion of $^{18}$F-Labeled Choline Analogs J Nucl Med 2007 48: 1225

3. Kwee SA, Thibault G, Stack R, Coel M, Furusato B, Sesterhenn I. Prostate Imaging with 18F-Fluorocholine Using a Whole-Body Positron Emission Tomograph. Nuclear Science Symposium / Medical Imaging Conference Institute of Electrical and Electronics Engineers 2007.

4. Park H, Kwee S, Thibault G, Stack G, Furusato B, Sesterhenn I, Meyer CR. Registration Methods for Histological Slides and *ex vivo* MRI of Prostate. Nuclear Science Symposium / Medical Imaging Conference Institute of Electrical and Electronics Engineers 2007.

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# Cancer Imaging With Fluorine-18–Labeled Choline Derivatives

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**The choline transporter and choline kinase enzyme frequently are overexpressed in malignancy. Therefore, positron-emitter-labeled compounds derived from choline have the potential to serve as oncologic probes for positron emission tomography. The fluorine-18 ($^{18}$F)–labeled choline derivative fluorocholine (FCH) in particular has demonstrated potential utility for imaging of a variety of neoplasms, including those of the breast, prostate, liver, and brain. The pharmacokinetics of FCH and other choline tracers allow for whole-body imaging within minutes of injection while still achieving high tumor-to-background contrast in most organs, including the brain. These features, along with the possibility of imaging malignancies that have proved elusive with the use of $^{18}$F-fluorodeoxyglucose positron emission tomography support further clinical investigations of $^{18}$F-labeled choline tracers.**

**Semin Nucl Med 37:420-428** 

In mammals, choline is an essential nutrient that serves as an extrinsic substrate for the synthesis of phosphatidylcholine (PC), a major constituent of the cell membrane. Phosphorylation by choline kinase (CK) constitutes the first intermediate step in the incorporation of choline into phospholipids via the Kennedy pathway. The importance of this metabolic pathway for cell viability is underscored by the fact that there are no known inherited diseases in humans affecting this pathway. However, in cancer, there is often an increase in the cellular transport and phosphorylation of choline, as well as an increase in the expression of the CK enzyme.[1–3] These observations have fueled interest in developing imaging and therapeutic agents out of compounds metabolized by CK. With this in mind, this article will summarize the development of fluorine-18 ($^{18}$F)-labeled choline radiopharmaceuticals as oncologic probes for positron emission tomography (PET).

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Supported by U.S. Department of Defense Prostate Cancer Research Program Grant PC04130, and National Institutes of Health/ National Cancer Institute grant CA108620.
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## Development of Choline Tracers Labeled With Fluorine-18

Tumor imaging with choline-based tracers was introduced by Hara and coworkers using carbon-11 ($^{11}$C) choline PET to successfully visualize brain tumors and prostate cancer.[4,5] As a true tracer, $^{11}$C choline is biochemically indistinguishable from natural choline. This compound has shown particular promise for imaging tumors of the genitourinary tract because of its limited urinary clearance and avidity for bladder and prostate cancers.[6–11] However, the short decay half-life of the carbon-11 (20 minutes) has limited its use to centers equipped with an on-site cyclotron.

The practical need for longer-lived agents has led subsequently to the development of $^{18}$F-labeled choline derivatives. The first of these, fluoroethylcholine (FeCH) and $^{18}$F fluorocholine (FCH), were introduced by Hara and coworkers and DeGrado and coworkers, respectively.[12,13] Contrary to initial observations, these compounds, which are phosphorylated by CK, do appear to participate further in the synthesis of membrane phospholipids as substrates for cytidylyltransferase, although the rate of their incorporation into phospholipids may be slower than that of choline.[14] In addition to compounds that serve as specific substrates of CK, there are choline transporter-specific ligands which can be

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doi:10.1053/j.semnuclmed.2007.07.003

used to specifically image the choline transporter system. These include deshydroxy-$^{18}$F fluorocholine as well as analogs of hemicholinium-3, an inhibitor of membrane choline transporter.[15,16] Of these agents, FCH has undergone the most study to date.

FCH is a fluoromethylated analog of choline consisting of fluoromethyl-dimethyl-2-hydroxyethylammonium labeled with fluorine-18. Several synthesis methods are available for producing this compound in commercially acceptable yields.[17–20] At the present time, it is not known which choline derivative is most advantageous for clinical use. There have been no direct in vivo comparisons between individual compounds, and previous in vitro comparisons have not controlled for potentially confounding factors, including the presence of synthetic contaminants such as dimethylethanolamine, which may modulate the phosphorylation and transport of these compounds in vitro.[21] In vitro experiments suggest that the rate of FCH phosphorylation by yeast CK, as well as the rate of FCH uptake by PC-3 cancer cells, does approach that of natural choline.[18] Thus, for now, it may be sufficient to consider FCH as a prototypical $^{18}$F choline until the optimal formulation is known.

Given the role of extrinsic choline in eukaryotic phospholipid synthesis, tracers derived from choline were proposed as imaging agents for measuring proliferative or mitogenic activity. The observation that phosphorylcholine can trigger DNA synthesis in quiescent NIH3T3 fibroblasts, along with the observation that inhibition of CK (by hemicholinium-3) can block proliferation activity unless bypassed by extrinsic phosphorylcholine, supports CK as a regulator of mitogenic activity.[22] However, to date, few studies have shown a strong correlation between markers of proliferation in malignant tumors and choline radiopharmaceutical uptake in vivo. A study that compared tumor uptake of $^{11}$C choline and ki-67 labeling in malignant prostate tissues did not support the presence of a direct correlation between $^{11}$C choline uptake and cellular proliferation rate.[23] One possibility is that transformation reduces the efficiency of choline metabolism, resulting in a disassociation of choline uptake rate and cell membrane synthesis rate. The general finding of high levels of phosphorylcholine in a variety of tumor types is consistent with the argument that these rates are not well-coupled. Despite the apparent avidity of choline tracers in a variety of malignancies, further research will be needed to determine whether clinically relevant markers of tumor growth can be derived from the measured uptake of these compounds.

The choline metabolite peak on magnetic resonance spectroscopy (MRS) also has been proposed as an indicator of malignancy or proliferation.[24] However, correlations between $^{18}$F-FCH or $^{11}$C choline uptake on PET and choline metabolite concentrations on MRS have not always been observed, alluding to the possibility that increased choline spectral peaks on MRS may not specifically reflect free choline or the active accumulation of choline metabolites by cells.[25,26] For example, in the case of a tumefactive demyelinating lesion, where increases in choline metabolite concentrations are frequently observed with MRS (presumably due to demyelination), there may not be corresponding increases in the uptake of $^{11}$C choline or $^{18}$F- FCH.[26,27] In malignant glial tumors, where mitogenic activity would be expected to result in the active utilization of choline, a direct regional correlation has been observed between $^{18}$F-FCH uptake and choline metabolite peaks on spectroscopy.[26] Without a better understanding of the biochemical basis of what is measured by both MRS and choline-based PET imaging, it will be difficult to integrate this information for clinical purposes.[24] Because there is occasional discordance, there will most likely be complementary value to both measures.

Because the authors of several in vitro studies have suggested the cellular uptake of FCH is dependent on choline transporter and CK activity, we explored the expression of these proteins in an array of 30 distinct tumor types. Abnormal expression of one or both proteins was observed in most malignancies, including prostate carcinoma, glial tumors, breast carcinoma, lymphoma, sarcoma, esophageal carcinoma, melanoma, and lung carcinoma. In the case of glial tumors and breast cancer, the degree of CK expression was found to correspond to tumor grade (Fig. 1). Pilot investigations with FCH-PET in a limited number of patients with these diseases have produced analogous results supporting the potential of radiolabeled choline metabolic substrates for imaging a broad variety of neoplasms (Fig. 2).

DeGrado and coworkers pursued further work to develop FCH as a clinical imaging probe. These investigators performed the first human dosimetry study of $^{18}$F-FCH to determine the dose-critical organ and radiation dose limit for research studies. While the favorable dosimetry of FCH and ability to perform scans shortly after injection allows for excellent image quality at commonly administered doses, newer PET instruments with very high count-rate performance will likely be capable of optimal image quality at a lower dose.[28]

## Prostate Cancer Imaging With Fluorocholine

Prostate cancer is the second-leading cause of cancer death in American men older than 50 years of age. Clinically, there has been a long-standing need for better imaging methods that can be applied to diagnose, risk stratify, stage, and direct treatments for prostate cancer. Conventional $^{18}$F-fluorodeoxyglucose (FDG)-PET has proven to be of limited usefulness for diagnosing prostate cancer, although it does appear possible to detect advanced or metastatic prostate cancer with this technique.[29,30] To compare the avidity of FCH and FDG for prostate cancer, Price and coworkers performed both FCH-PET and FDG-PET in 18 patients with prostate cancer.[31] They found that more lesions were identifiable with FCH-PET, including lesions of the prostate, bone, and soft tissues. In addition, an in vitro component to this study revealed significantly greater uptake of FCH compared with FDG in cell cultures of androgen-dependent (LnCAP) and -independent (PC-3) prostate cancer. These results favor the use of FCH rather than FDG for prostate cancer imaging.

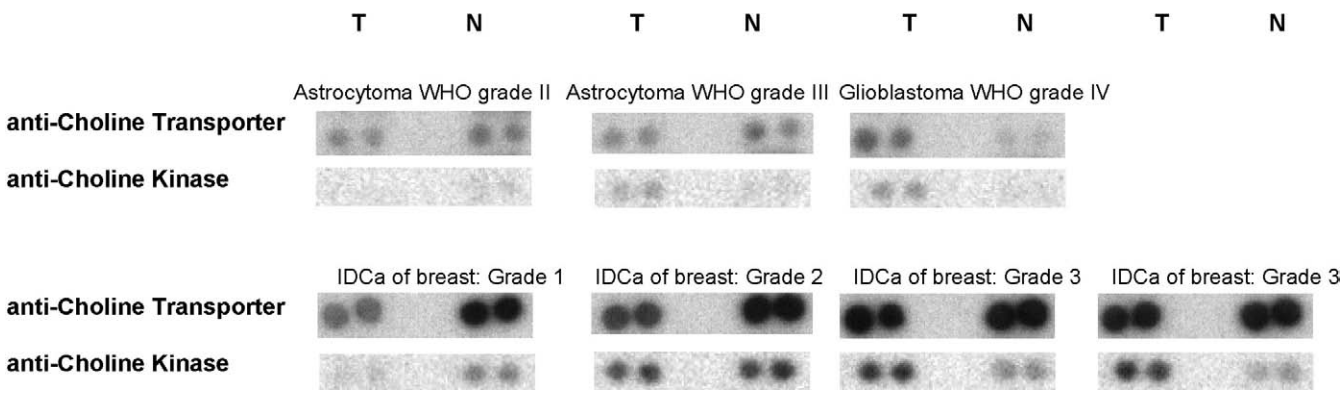


**Figure 1** Dot blot analysis of choline transporter and choline kinase (CK) expression in tumor lysates (T) of glial tumors and breast carcinomas and their corresponding normal (N) tissue controls. Washing conditions were optimized for CK expression by use of Western blot standards. Top row: CK expression (and, to a less-appreciable extent, choline transporter expression) increases with increasing World Health Organization tumor grade in malignant glial tumors. Bottom Row: Both choline transporter and CK expression increases with increasing breast cancer grade. Malignant expression of CK exceeds that of normal breast tissue, but normal tissue expression of choline transporter exceeds that of malignancy. IDCa, infiltrating ductral carcinoma.

Subsequently, a number of early clinical studies have investigated the potential usefulness of FCH-PET for diagnosing or localizing primary prostate cancer. Currently, ultrasound-guided prostate biopsy is the most common method for diagnosing this disease. However, conventional prostate biopsy using standard 6 or 12 needle templates is susceptible to sampling error, with a false-negative rate as high as 20% regardless of the number of needles used.[32,33] A few studies have preliminarily investigated FCH-PET as a method for improving cancer localization in the prostate. In a study by Kwee and coworkers, [34] prostate sextants harboring malignancy were found to demonstrate significantly higher FCH uptake than biopsy-negative sextants, with the cancer-affected side in 6 of 6 patients with unilaterally positive prostate biopsies demonstrating the highest uptake on FCH-PET. By identifying areas within the prostate that have the highest likelihood of malignancy, FCH-PET could potentially serve to identify areas for additional biopsy, thus potentially reducing the false-negative rate of the procedure.

It is worth noting that, with FCH-PET, delayed imaging may be required for adequate tracer uptake and distribution in the prostate. In a study by Kwee and coworkers, delayed FCH-PET imaging up to 1-hour after injection led to both a significant increase in measured uptake by malignant tumors in the prostate, as well as a significant decrease in uptake in benign areas.[35] In contrast, FCH-PET imaging of the prostate at 2 minutes after injection was not found to be useful for differentiating between benign hyperplasia and malignancy in the prostate.[36] Additional studies using whole-prostate specimen analysis for histopathologic correlations are underway to better estimate the accuracy of intraprostatic cancer localization with FCH-PET.

The advent of hybrid PET/computed tomography (CT) has led to a number of studies using FCH for whole-body staging of prostate cancer.[36–39] Because of its ability to provide structural/anatomical correlation, PET/CT is advantageous for localizing disease in lymph nodes, a common route of spread in prostate cancer. In a study by Schmid and coworkers,[36] FCH PET/CT was able to identify local and distant sites of disease in both patients with newly diagnosed prostate cancer and patients suspected of having recurrent cancer. Lesions that were identified included local recurrent tumors, nodal metastases, and skeletal metastases. Thus, FCH PET/CT may have the potential to provide information that can be used for deciding between regional and systemic treatment in both patients with newly diagnosed and recurrent prostate cancer.

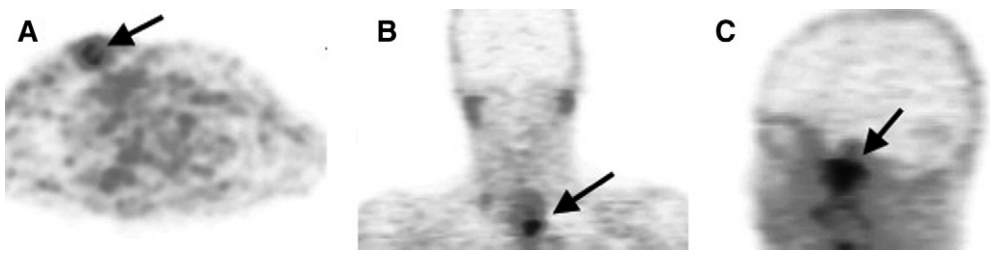


**Figure 2** FCH-PET scans in patients with malignant breast, esophageal, and nasopharyngeal cancers. (A) Transaxial PET image: increased FCH uptake in right breast carcinoma (arrow). (B) Coronal PET image: increased FCH uptake in esophageal carcinoma. (C) Saggital PET image: increased FCH uptake in recurrent metastatic nasopharyngeal carcinoma.

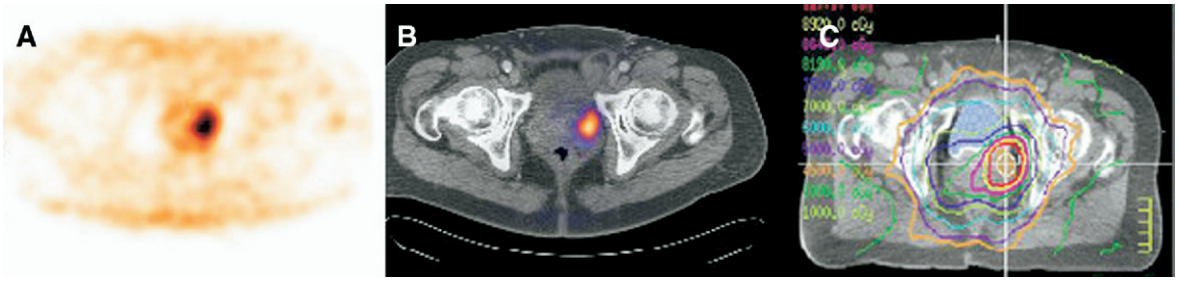


**Figure 3** FCH-PET-guided radiotherapy. (A) The black area on this transaxial PET image corresponds to increased FCH uptake in a malignant tumor situated in the left lobe of the prostate gland. In an experimental treatment plan, this PET-defined biological target volume (BTV) will be prescribed a radiation dose of at least 90 Gy. (B) A "fused" PET/CT image is used to plan the radiation treatment. In this patient, the prostate volume was 62 mL and the BTV volume was 14 mL. (C) The experimental treatment plan is summarized by colored lines corresponding to prescribed iso-doses of radiation. Doses of 91 Gy and 76 Gy were prescribed to the BTV and prostate, respectively, without significantly increasing radiation exposure to un-involved organs (rectum and bladder).

With regard to recurrent prostate cancer, several other studies using FCH-PET/CT have explored the relationship between prostate-specific antigen (PSA) level and lesion detection in patients with posttreatment increases in PSA. A study by Heinisch and coworkers found that FCH PET/CT detected tumor recurrences in only half of patients suspected of having recurrent prostate cancer with a PSA level <5 ng/dL.[38] In another study of 100 patients by Cimitan and coworkers, use of FCH-PET/CT led to the identification of prostate cancer recurrence in 53 of the patients.[39] In this study, 89% of the patients with presumably false-negative FCH-PET/CT scans had a serum PSA level <4 ng/dL. Thus, FCH-PET/CT does appear to be less sensitive for recurrent prostate cancer if the PSA level is low. However, it is worth noting that the use of FCH-PET in these studies had value for distinguishing between local and distant metastatic recurrence. Such a distinction is clinically relevant since it helps determine the appropriateness of local salvage therapy.

With regard to clinical decision making and treatment planning, FCH-PET/CT may also have value for newly diagnosed patients with prostate cancer.[34,36,40] Recently, Langsteger and coworkers[40] reported that FCH-PET/CT performed during initial preoperative staging for prostate cancer patients who were at high risk for metastases (eg, Gleason score >7 or PSA >10 ng/mL or doubling time <3months) led to downstaging in 4% of cases and upstaging in 12% with potential consequential changes in clinical management. In addition to staging, FCH PET/CT could also prove useful for planning treatments for patients with newly diagnosed prostate cancer. For example, with intensity-modulated radiation therapy, or a combination of brachytherapy and external radiation therapy, it may be possible to apply very high radiation doses to specific targets in the prostate identified with FCH PET, while still treating the remainder of the prostate with a conventional therapeutic radiation dose in addition to maintaining acceptable levels of radiation exposure to uninvolved organs. The current conventional approach is to treat the prostate uniformly with a radiation dose that is usually limited by toxicity concerns rather than the radiation sensitivity of the tumors. By targeting tumor areas specifically, it becomes possible to apply higher doses to the most critical targets while still maintaining a reasonably safe level of radiation exposure to uninvolved areas. We examined the feasibility and safety of this concept by applying FCH-PET to guide the augmentation of radiation dose to prostate tumors. An experimental intensity-modulated radiation therapy protocol was developed to deliver the highest radiation dose (at least 90 Gy) to a biological target volume (BTV) that corresponds to the area of highest $^{18}$F-FCH uptake in the prostate. This protocol was based on the premise that the area of highest tracer uptake on prostate PET images represents the dominant area of malignancy in the prostate.[34,35,41] While treating the BTV with supra-conventional radiation doses, the experimental treatment protocol was still designed to maintain a tolerable dose to surrounding normal tissues while achieving a conventional therapeutic dose of 76 Gy to the remainder of the prostate gland. In this manner, the experimental treatment would be expected to be at least equivalent to conventional treatments with regards to potential efficacy. Using standard dose volume histograms to estimate the radiation exposure to uninvolved organs (rectum and bladder), we were able to preliminarily assess the potential safety of both experimental and conventional treatment plans.

Figure 3 illustrates the experimental treatment approach. In this plan, a dose of 91 Gy could be delivered to the BTV while attaining a minimum dose of 76 Gy to the remainder of the prostate. This experimental plan also met desired safety constraints, with less than 20% of the rectal wall receiving 70 Gy or higher dose and less than 25% of the bladder volume receiving 75 Gy or higher dose. Although not actually used, this plan demonstrates the feasibility of selective radiation dose escalation using FCH-PET as a means to target high-risk intraprostatic regions, while still achieving therapeutic goals for the remainder of the prostate and meeting the safety constraints of other organs. Given that the likelihood of local tumor control after external beam radiotherapy for organ-confined prostate cancer is directly related to radiation dose, this approach may potentially improve the therapeutic efficacy of radiation therapy, while maintaining an acceptable safety profile. A clinical trial will ultimately be required to

evaluate the clinical benefit of this approach in a suitable number of patients.

## Brain Imaging

DeGrado and coworkers first reported brain tumor imaging with $^{18}$F-FCH in a patient with biopsy proven recurrent anaplastic astrocytoma.[18] These investigators noted that the low concentration of FCH in normal cerebral cortex allowed for excellent delineation of the tumor from normal brain. They observed that a ~10:1 tumor-to-cortex ratio was achievable within 5-minutes of tracer injection. FDG-PET revealed a corresponding area of increased FDG uptake; however, the tumor boundaries were difficult to assess with FDG because of high uptake by normal cortex. Although overall there is very little uptake of FCH in the brain, it is worth noting that physiologic uptake does occur in the pituitary gland and choroids plexus. Physiologic uptake in these areas should not be difficult to recognized, especially if the FCH-PET images are interpreted in conjunction with brain magnetic resonance imaging (MRI).

The use of FCH-PET to evaluate primary and metastatic brain tumors was investigated subsequently by Kwee and coworkers in 30 patients with solitary brain lesions.[42] This study found that high-grade gliomas, brain metastases, and benign lesions could be distinguished on the basis of measured FCH uptake, with metastases demonstrating significantly greater uptake than high-grade gliomas. Furthermore, high-grade gliomas were distinguished in this study by a characteristic pattern of FCH uptake consisting of increased FCH uptake beyond the areas of contrast enhancement on MRI (Fig. 4). This pattern of "peritumoral uptake" is hypothesized to be due to infiltration of the white-matter tracts by malignant cells. Such a process of occult tumor spread is known to occur frequently in high-grade gliomas but seldom in metastases.[43–46] This study also found that lesions with low FCH uptake were likely to remain stable radiographically at 1-year of follow-up. However, because this study did not include low-grade tumors, further investigations in a broader spectrum of patients are warranted to evaluate the diagnostic and prognostic value of this technique in patients presenting with an intracranial mass.

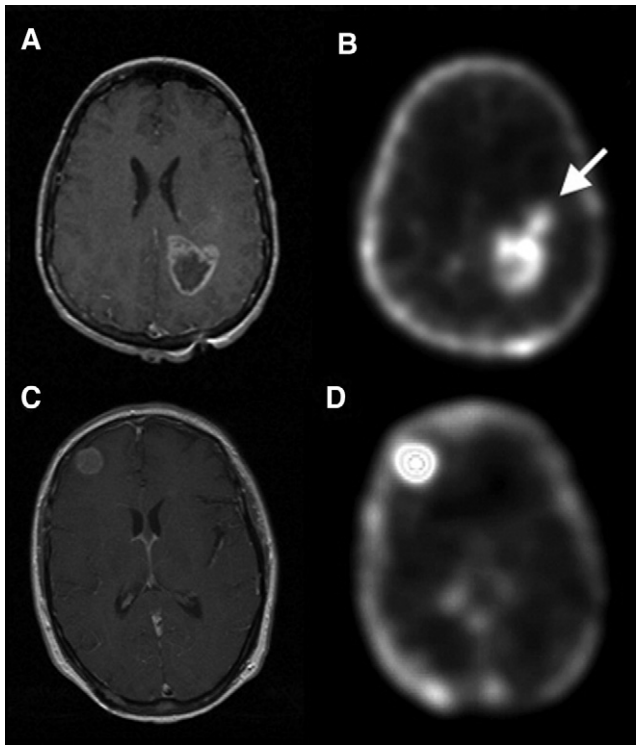


**Figure 4** MRI and FCH-PET in primary (A and B) and metastatic (C and D) brain tumors. (A) Glioblastoma multiforme observed as a ring-enhancing lesion in the left occipital lobe on T1-weighted MRI. (B) The corresponding area on PET shows increased FCH uptake. However, abnormal uptake (arrow) was also noted anteriorly beyond the area of enhancement. This characteristic ("peritumoral uptake") appears specific to high-grade gliomas. C) Metastatic ovarian carcinoma seen as a ring-enhancing lesion in the right frontal lobe on T1-weighted MRI. (B) In contrast to the previous case, increased FCH uptake on PET corresponds only to the region of contrast enhancement on MRI.

## Liver Imaging

Hepatocellular carcinoma (HCC) is the fifth most frequent cancer worldwide and the most frequent cause of death in cirrhotic patients.[1] The sensitivity of FDG-PET for the detection of HCC is suboptimal, ranging between 50% and 70%.[3,4] Given that the use of MRS demonstrates high choline content in HCC, it may be possible to detect this disease using FCH,[7] despite the fact that the liver demonstrates significant physiologic uptake of FCH. A proof of concept study was performed by Talbot and coworkers, comparing FDG PET/CT with FCH PET/CT in 9 patients known to have HCC.[8] All 9 patients were positive with FCH (100%) in contrast to 5 with FDG (56%). Despite significant FCH uptake by unaffected portions of the liver, HCC lesions as small as 9-mm in size could be distinguished visually, and by semiquantitative uptake measurement, on FCH PET/CT (Fig 5 and 6). A trend for greater uptake of FCH in well-differentiated HCC compared with moderate and poorly differentiated HCC also was observed in this study. In 2 cases of metastatic HCC, FCH was also taken-up by distant metastases to the lungs and bone. In contrast, colorectal carcinoma metastatic to the liver was found to demonstrate low uptake of FCH relative to the liver, thus potentially distinguishing these lesions from those of HCC (Fig. 7). However, the lack of uptake by metastatic colorectal carcinoma does suggest that FCH PET may be insensitive for colorectal carcinoma and possibly other types of metastases. A subsequent study is currently underway in patients with liver masses to prospectively compare the relative accuracies of FCH PET/CT and FDG PET/CT for the diagnosis, staging, and localization of liver tumors.

## Practical Issues and Potential Pitfalls in FCH-PET Imaging

FCH is effectively cleared from the blood within minutes after its intravenous administration. During this brief period

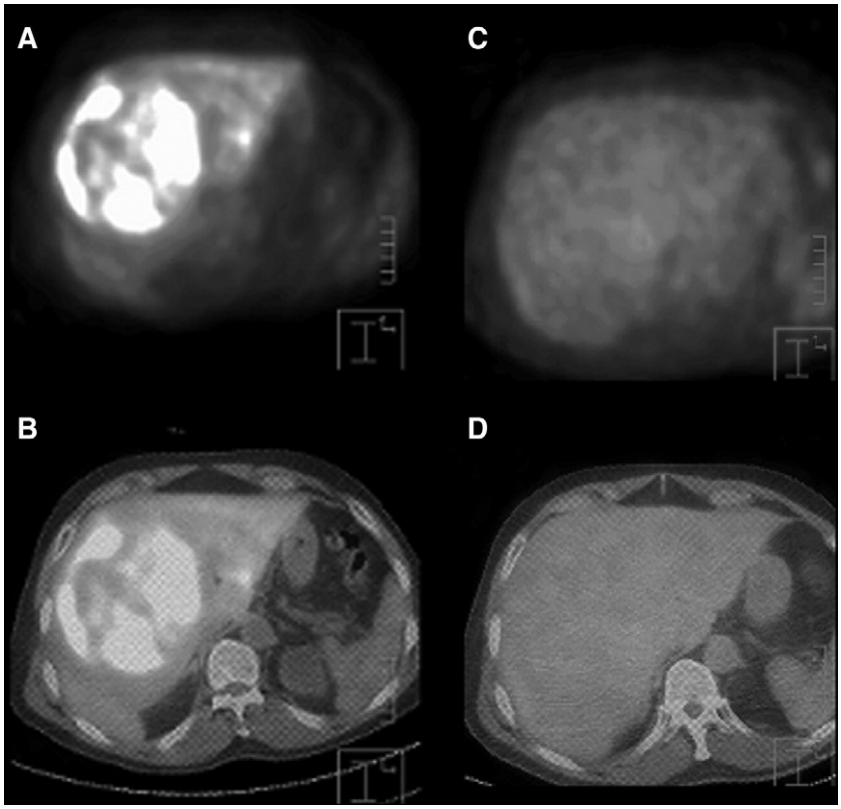


**Figure 5** Large liver mass in a patient with hepatitis C virus infection. A liver lesion with intense FCH uptake (maximum standardized uptake value = 20) was noted on PET (A). The lesion measures 12.5 cm and involves Couinaud segments VI and VIII on FCH PET/CT (B). No abnormal uptake is noted on FDG PET (C) and PET/CT (D). Grade 2 well-differentiated hepatocellular carcinoma was confirmed after surgery.

of rapid clearance, FCH is capable of significant uptake and retention by malignant tumors, while achieving minimal retention in organs such as the heart and brain. These features of FCH are advantageous, because they allow for very efficient scanning of the entire body. However, as a consequence of rapid blood clearance, the tissue distribution of FCH is likely to be dependent on delivery (ie, blood flow). Although a study has not been done to correlate blood flow and choline uptake in a tumor model, the correlation is believed to be high since there is little redistribution from other organs after clearance from the blood, and the metabolism of FCH to noncholine metabolites leaves no alternative mechanism for accumulation in tumors. Thus, the influence of blood flow on FCH distribution should be carefully considered in image interpretations, particularly if FCH PET is used to monitor an intervention that modulates blood flow.

A practical end result of the rapid in vivo kinetics of FCH is a whole-body PET scan that can be completed shortly after tracer injection. However, rapid clearance of background activity necessitates the existence of a normal process by which the tracer is eliminated from circulation. In the case of $^{18}$F-FCH, this process depends on physiologic tracer uptake by the kidneys. Thus, like with FDG, there can be significant accumulation of radioactivity in the urine over time. This activity has the potential to obscure malignant lesions near the genitourinary tract. However, based on collective experiences in patients with prostate cancer, the urinary excretion of FCH has seldom caused problems with image interpretation.[31,34,35,38,39] This may be caused in part by the effects of urodynamic alterations, which are common in men with prostate disease. Conditions such as urinary retention may serve inadvertently to reduce the concentration of radioactivity in the bladder through dilution effects. In addition, PET imaging protocols with FCH have the flexibility to acquire images during a time when bladder radioactivity is not high.[34,36] For example, using a dynamic scan acquisition, it is possible to retrospectively sum the frames acquired just before the appearance of urinary radioactivity to provide a pelvic image of early FCH distribution. Although hydration or fluid restriction have been proposed as a means to influence urinary excretion, we have not been able to observe a certain effect of these interventions on the appearance of urinary radioactivity. The influence of diet or fasting on the biological distribution of FCH is also not known. Although bladder catheterization and irrigation can effectively eliminate artifacts from bladder radioactivity,[47] we have not routinely used this technique in studying patients with prostate cancer because these patients may be at increased risk of complications after bladder catheterization. Finally, with regards to avoiding potential interpretive pitfalls, the use of PET/CT has helped tremendously by providing anatomical references for findings in the retroperitoneum and pelvis.

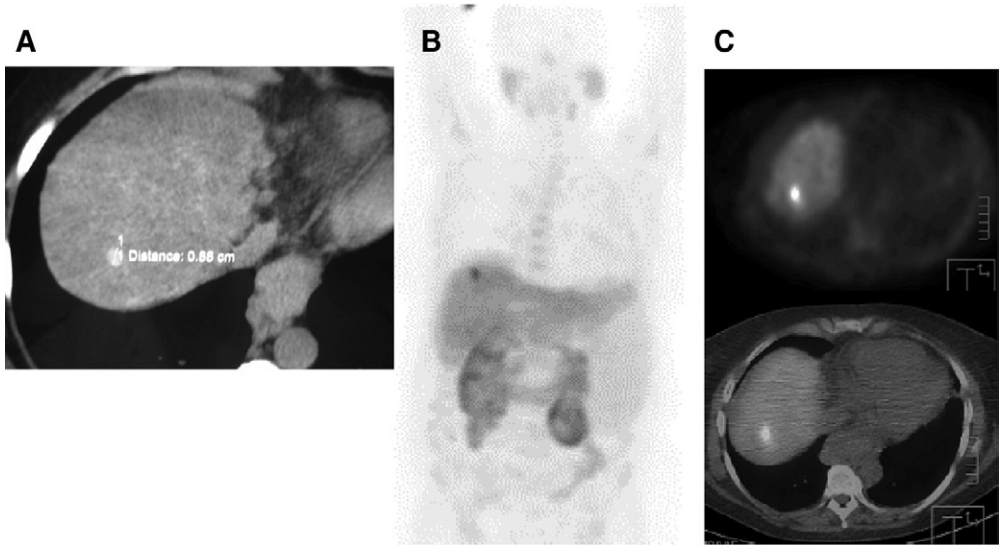


**Figure 6** Recurrent hepatocellular carcinoma in a patient with alcoholic cirrhosis. Serum alpha-foeto protein levels were elevated at 1,250 ng/mL. On CT, a 9-mm nodule was found in the segment VII of the liver (A). The lesion demonstrated significant FCH uptake relative to surrounding liver, as shown on maximum intensity projection image (B) and corresponding FCH PET (C, top) and PET/CT images (C, bottom).

Uptake in benign conditions such as infection is known to limit the specificity of FDG-PET for malignancy. It remains plausible that inflammation or benign proliferative processes can lead to an increase in FCH uptake, thus also reducing the specificity FCH-PET for malignancy. It is already known that some benign conditions can cause a transient increase in FCH uptake. Price and coworkers has reported that benign inguinal lymph nodes can demonstrate transiently increased FCH uptake during the first 5 minutes of injection, but that uptake within these benign lymph nodes diminishes rapidly to background levels by 20 minutes.[31] We have also observed this "washout" phenomenon in other lymphatic regions, including cervical lymph nodes. Thus, as with the prostate,[35] delayed or dynamic imaging may be required to resolve issues of transient increases in uptake in benign tissues.

It remains possible that benign proliferative conditions can lead to a persistent increase in choline tracer uptake. With $^{11}$C choline, persistent increases in uptake have been observed in cases of liver regeneration posthepatectomy, proliferative synovitis, and inflammatory lung nodules.[48–50] Whether the same holds true for $^{18}$F-FCH is not known at this time. In one animal model, FCH uptake in a sterile inflammatory lesion was much less than uptake in an implanted tumor, whereas the uptake of tritium-labeled deoxyglucose was relatively increased in both types of lesions.[51] Other experimental models suggest tissue inflammation (caused by infection or acute radiation injury) can lead to measurable increases in FCH uptake,[17,52] whereas blood–brain barrier disruption alone in the absence of inflammation (as modeled by cryolesions) does not.[17] In lesions caused by radiation injury, the uptake of FCH is lower than uptake in malignant tumors, supporting FCH as potentially useful for distinguishing tumor recurrence from necrosis after radiation therapy.[17] Further in vivo studies in a wider spectrum of diseases are needed ascertain the diagnostic specificity of

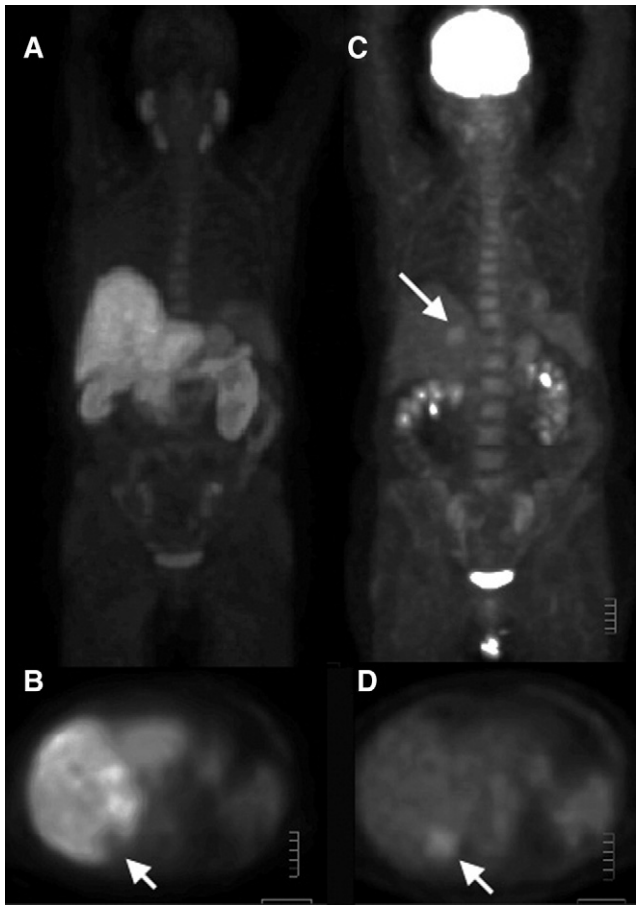


**Figure 7** FCH PET (A and B) and FDG PET (C and D) images of a hepatic mass in a patient with a previous history of rectal adenocarcinoma. The mass (arrow) took up FDG and not FCH, appearing photopenic on FCH PET as compared with the surrounding healthy liver tissue. The lesion was confirmed to be metastatic rectal adenocarcinoma at surgery.

FCH PET when applied to the evaluation of tumors and tumor recurrences.

## Conclusion

The $^{18}$F-labeled choline analogs, and in particular FCH, are currently under investigation as oncologic probes for the detection and monitoring of malignancies. These probes may be viewed as in vivo biomarkers of choline transporter and CK activity, although their uptake may also reflect a component of tissue perfusion. To date, the majority of studies have focused on the use of the FCH PET to evaluate prostate cancer, with preliminary studies having provided encouraging results for detecting primary and metastatic cancer. Experience in other tumor types is growing, including work involving brain and liver tumors. In most organs, high tumor-to-background contrast is achieved with FCH within minutes of injection. Excellent discrimination can be achieved in the brain, where there is very little physiologic uptake of FCH. In other organs such as the liver, malignant discrimination appears still possible despite a moderate degree of physiologic uptake. The rapid circulatory clearance of FCH is advantageous from a practical point of view since it allows completion of a PET scan within minutes of tracer injection. Although the renal excretion of FCH is not ideal for evaluations of the urinary tract, it has not proven intractable in actual practice. Therefore, FCH and other choline derivatives may possess the features of efficiency and ease of use that is important for successful clinical application.

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## The Impact of Urinary Excretion of $^{18}$F-Labeled Choline Analogs

**TO THE EDITOR:** We have read with interest the article of Schuster et al. (*1*) on the initial evaluation of an $^{18}$F-labeled amino acid transport tracer, anti-1-amino-3-$^{18}$F-fluorocyclobutyl-1-carboxylic acid (FACBC) in patients with prostate cancer. In this preliminary study on a small cohort of patients, FACBC appeared to have several favorable properties for the imaging of prostate cancer in the pelvic region, including avid uptake in primary tumors and metastases in lymph nodes and bone, relatively lower uptake in nonmalignant tissues of the prostate or lymph nodes, and low urinary excretion. The results showed a certain promise that the evaluation of amino acid transport function with this tracer may be useful in new and recurrent prostate cancer.

However, we would like to respond to the comments of the authors that imaging of prostate cancer with $^{18}$F-labeled choline (FCH) is disadvantaged because of its relatively higher urinary excretion pattern. The urinary excretion of FCH has been reported to be 4.9% ± 4.8% of the administered dose in female patients and 1.9% ± 1.6% in male patients within the first hour after injection (*2*). Because of the extremely rapid renal clearance of FCH, most of the urinary radioactivity generally arrives at the urinary bladder within the first 20 min. Although urinary activity has the potential to confound the imaging of prostate cancer, image acquisition protocols have been designed to minimize the impact of this potential problem. Dynamic imaging over the pelvic region for the first 10 min after injection allows clear delineation of tumor uptake that precedes the appearance of radioactivity in the ureters and bladder (*3,4*). Consequently, it is possible to retrospectively exclude frames that show significant urinary interference. Furthermore, because there is rapid circulatory and urinary clearance of tracer but little washout from malignant tumors, voiding followed by delayed scanning with or without gentle hydration can also lead to satisfactory prostate images with high tumor-to-background contrast (*5*). The dynamic imaging information is useful not only for exclusion of urinary radioactivity but also for understanding the relationship of early FCH uptake, indicative of tracer delivery (perfusion) and choline transport, and of later tissue retention that is dependent on intracellular metabolism. In this regard, Schuster et al. (*1*) also found dynamic imaging to provide important information on FACBC kinetics: The amino acid analog was found to be transported but not metabolically trapped. Thus, the relative advantage of the lower urinary excretion of FACBC diminishes as the tracer washes out of malignant regions. The use of FACBC for whole-body imaging may require short image acquisition protocols, which may limit detection sensitivity for tumors. It will be of high interest to understand how rates of amino acid transport in prostate cancer, as seen with FACBC, relate to rates of choline transport and choline kinase activity, as seen with positron-labeled choline analogs.

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5. Kwee SA, Wei H, Sesterhenn I, Yun D, Coel MN. Localization of primary prostate cancer with dual-phase $^{18}$F-fluorocholine PET. *J Nucl Med.* 2006;47:262–269.



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DOI: 10.2967/jnumed.107.040667

## ABSTRACT

### Prostate Imaging with 18F-Fluorocholine Using a Whole-Body Positron Emission Tomograph

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**Abstract:**

Non-invasive methods for localizing malignancy in the prostate gland are needed for targeted biopsy or treatment. Due to its anatomical location, the prostate is a challenging organ to image with nuclear techniques. We evaluated whole-body positron emission tomography (PET) with the investigational tracer fluorine-18 fluorocholine for sextant localization of prostate tumors in patients with histologically confirmed prostate cancer. Step-section histopathology of whole-mounted radical prostetectomy specimens served as the reference for comparisons with pre-operative PET of the prostate. PET images were obtained using a 32-ring PET device 10 minutes after the intravenous administration of 3.3 to 4 Mbq/kg of fluorine-18 fluorocholine. The maximum standardized uptake value (SUVmax) of prostate sextants was measured by region of interest analysis. Sixty-one of 90 prostate sextants were found to contain malignant tumors. The mean total tumor volume per specimen was 4.9 cc (range 0.01 cc to 28.7 cc). Mean SUVmax was 6.0 in malignant sextants and 3.8 in benign sextants ($p < 0.0001$). The area under the receiver operating characteristic (ROC) curve was 0.82 for sextant detection of malignancy based on SUVmax measurement. There was a statistically significant correlation between maximum tumor diameter and sextant SUVmax in malignant sextants (Pearson correlation coefficient $r=0.54$, $p < 0.05$). In 13 subjects, the sextant with the highest SUVmax contained the largest tumor for that specimen. Six of 9 falsely-negative sextants contained only tumors with diameters smaller than 0.5 cm. In conclusion, prostate SUVmax is a reproducible, semi-quantitative measure of fluorocholine uptake that can be used to localize dominant malignant tumors in the prostate. However, small prostate tumors can elude detection despite the availability of tumor-specific tracers. This data may be of value to phantom and simulation experiments directed at optimizing PET for prostate imaging.

**Acknowledgements:** This work is supported by U.S. Army Medical Research and Materiel Command grant W81XWH-05-1-0056.

**Summary:**

Prostate cancer is the second leading cause of cancer death in American men over 50 years of age. Invasive ultrasound-guided prostate biopsy is the most common method for diagnosing prostate cancer. Unfortunately, this technique is prone to sampling error despite the use of various biopsy strategies. A technique for identifying areas in the prostate with the highest likelihood of malignancy would be advantageous in refining the biopsy strategy.

Positron emission tomography (PET) can detect tumors through measurement of metabolic changes at the cellular level. This technique works by depicting the biochemical interactions of radiolabeled tracers in the body. Unfortunately, fluorine-18 fluorodeoxyglucose, the only FDA-approved PET tracer for detecting cancer, is not useful for prostate cancer. We are the first site in the United States to be conducting clinical investigations with the fluorine-18 labeled PET tracer fluorocholine under its IND (investigational new drug) status with the FDA. The phosphorylation of fluorocholine by choline kinase (CK), an enzyme commonly over-expressed in malignancy, leads to the intracellular accumulation of this compound in malignant tissues *(1)*. The observation that there is increased choline metabolism in malignant prostate tissue relative to normal tissue supports the possibility of using fluorocholine PET to visualize cancer in the prostate gland *(2, 3)*.

In a histopathological correlation study, we assessed the diagnostic performance of a standard full-ring PET scanner using fluorine-18 labeled fluorocholine for the task of localizing malignant tumors in prostate gland sextants. The convention of prostate sextants is based on a biopsy template dividing the prostate into basal, mid, and apical portions on each side. To evaluate sextant-level prostate tumor detection, we performed histopathologic analysis on completely embedded whole-mounted prostate specimens from 15 patients who underwent PET imaging with fluorine-18 fluorocholine before prostatectomy. Written informed consent was obtained from all subjects prior to involvement in this institutional review board approved study.

Images were obtained with a 32-ring whole-body PET scanning instrument (SHR-22000, Hamamatsu Photonics KK, Hamamatsu City, Japan) using a fluorocholine protocol developed previously at our institution *(4)*. Measurement of uptake in prostate sextants was performed on computed tomography (CT)-registered PET images. For analysis, the prostate was manually segmented into sextants consisting of an upper (basal) one-third, middle one-third, and lower (apical) one-third portion on each side. Using region of interest (ROI) analysis, the maximum standardized uptake value (SUVmax) corresponding to each sextant was measured and recorded. SUVmax is a measure of radioactivity concentration defined as the maximum measured radioactivity divided by the injected radioactivity normalized to body weight. Two independent readers (S.A.K., M.N.C.) obtained concordant SUVmax measurements in all subjects. Histology of the prostate specimen was performed by the step-section technique. Areas of malignant tumor on each slide were assigned to their corresponding sextant by a pathologist with extensive experience in genitourinary pathology (I.A.S.). Sextants were classified as malignant if they contained at least one malignant tumor.

Sixty-one of 90 prostate sextants contained at least one malignant tumor on analysis of the whole-mounted specimen. The mean total tumor volume was 4.9 cc (range 0.01 cc to 28.7 cc). Mean SUVmax in malignant sextants was significantly higher than in benign sextants (6.0 vs. 3.8 respectively, $p < 0.0001$). The area under the receiver operating characteristic (ROC) curve was 0.82 for detection of sextant malignancy based on SUVmax measurement. Using a SUVmax of greater than 5.6 to classify malignancy, the sensitivity and specificity of PET was 64% and 90% respectively. Diagnostic sensitivity increases and specificity decreases with a lower classification threshhold. For example, using a SUVmax threshold of 4.0, the sensitivity and specificity of fluorocholine PET for sextant diagnosis was 85% and 62% respectively. The area of highest SUVmax in the prostate was localized to a malignant sextant in all subjects. In 13 out of 15 subjects, the sextant with highest SUVmax also contained the largest malignant tumor of that specimen. With a SUVmax classification threshold of 4.0, six out of 9 false-negative sextants contained only tumors with a diameter less than 0.5 cm. There was a statistically significant correlation between maximum tumor diameter and SUVmax in malignant sextants (Pearson correlation coefficient $r=0.54$, $p < 0.05$).

Several points warrant discussion. The use of an objective measure (SUVmax) to isolate prostate malignancy is more reproducible than subjective visual interpretation as commonly performed in radiology. Although tumor burdens were relatively low in our patients, the measurement of SUVmax in the prostate on fluorocholine PET still demonstrated an accuracy for sextant localization of prostate cancer comparable to that of MRI, a technique which requires visual interpretation *(2, 5)*. However, the finding of a correlation between

tumor diameter and SUVmax in malignant sextants suggests that the use of a single SUVmax threshold does limit the sensitivity of fluorocholine PET for identifying sextants harboring only small tumors.

The prostate is a challenging organ to image using nuclear techniques due to its small size and anatomical location (Figure 1). The intrinsic ability of PET to image small lesions is limited by the effects of radioactive scatter and positron travel. Furthermore, SUV is a voxel-based measure, and thus represents an assessment of mean radioactivity in a fixed volume of tissue. Assuming that fluorocholine is more concentrated in malignant cells, it would be expected that the measured SUV of a volume containing both malignant and benign cells would be lower than the measured SUV of a volume containing only malignant cells. Clinically speaking however, large tumors lead to greater morbidity, causing more symptoms and having a higher potential to metastasize. Thus, in light of the potentially favorable prognosis associated with smaller prostate tumors, a diminished sensitivity for small lesions may be acceptable to physicians, or even desirable, especially in frail patients in whom the risks of treatment could potentially outweigh the benefits.

Nevertheless, advances in PET imaging technology, and work towards developing prostate-optimized PET imaging devices, have the potential to capitalize further on prostate cancer-avid tracers such as fluorocholine. Recently introduced PET techniques such as time-of-flight imaging and dynamic scanning with list-mode data acquisition could significantly enhance the per-sextant or per-lesion evaluation of the prostate with PET. For evaluating organs such as the prostate, a substantial increase in PET count-rate performance (as can be achieved with time-of-flight) can lead to significantly improvements in PET image quality, especially in larger patients*(6)*. Data from our study on fluorocholine metabolism in prostate tumors and their surrounding organs may have value in future phantom and simulation experiments with the goal of optimizing PET for prostate imaging.

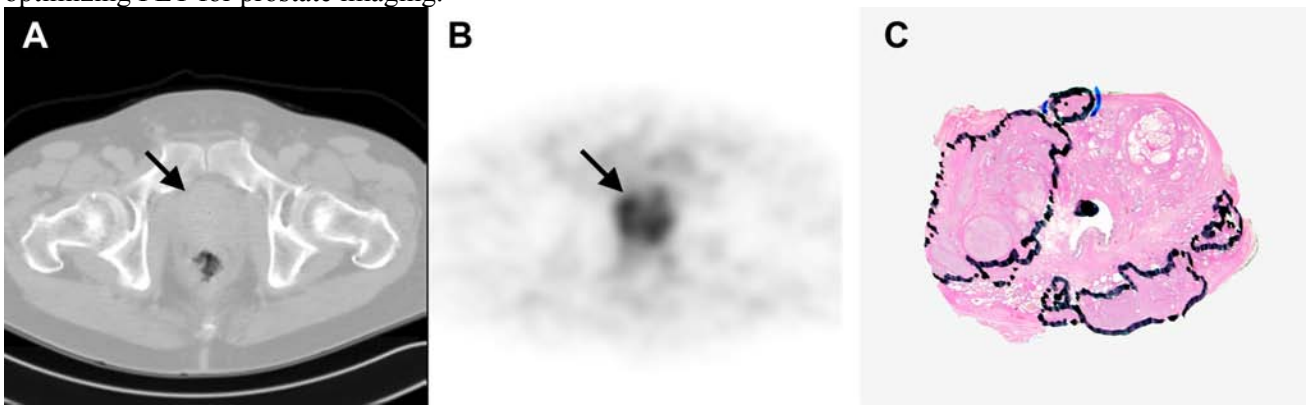


Figure 1. A. Computed tomography (CT) image of the pelvis at the level of the prostate. The prostate is a homogeneous structure in the pelvis center (arrow). Although there is prostate cancer, the prostate does not appear abnormal on the CT. B. Fluorocholine PET corresponding to the same level in the pelvis as the CT shows abnormally increased fluorocholine uptake in the peripheral zones of the prostate gland (arrow). C. After surgery, the corresponding prostate specimen was examined histologically. This 10X micrograph reveals multiple foci of prostate cancer (outlined in black), which regionally correspond to the abnormal areas of increased uptake on PET as shown in panel B.

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# Registration Methods for Histological Slides and *ex vivo* MRI of Prostate

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**Abstract:**

A methodology for registering histological slides and *ex vivo* MRI of prostate is proposed. After such registration is performed, spatial correspondence between histology slides and *ex vivo* MRI is established, thus histological truth can be propagated to the *ex vivo* MRI space. We employ the well established registration approach based on mutual information (MI) and thin-plate splines (TPS), which is automatic after user's initial placement of control points. Directly registering histology slides onto *ex vivo* MRI is challenging because 1) it is a difficult 2D (slice) to 3D (volume) registration problem and 2) there is a big difference in information content as histology slides are typically taken at much higher resolution than *ex vivo* MRI. Here we propose to overcome this challenge by breaking the difficult direct registration task into easier registration sub-tasks. For this purpose, we acquire digital photographs of prostate specimen as it is sectioned, which are referred to as block face photos. First, we register histology slides onto block face photos and then register block face photos onto *ex vivo* MRI. Results from two registrations tasks are combined to establish registration between histology slides and *ex vivo* MRI. Before the second registration task, we stack the block face photos into a volume so that registration onto the *ex vivo* MRI is a more stable 3D (volume) to 3D (volume) registration.

**Keyword:** registration, histology slides, prostate, *ex vivo* MRI.

**Acknowledgements:** This work is supported in part by NIH grant 1P01CA87634, U.S. Army Medical Research and Materiel Command grant W81XWH-05-1-0056, and NIH/NCI grant R41CA110121.

## 1. Introduction

The goal of registration is to establish spatial correspondence between two scans so that both can be viewed in the same spatial frame. Here we study the registration between histology slides and *ex vivo* MRI of prostate. This registration has two important applications; 1) If the registration process is repeated for many patients' prostates, one can build an atlas of prostate cancer sites, which can give you a quantitative probability of cancer presence given a location in the prostate. 2) It can be a bridge to establishing registration between *in vivo* MRI and histology slides. Combined with the registration of *in vivo* MRI and *ex vivo* MRI, one can establish registration between *in vivo* MRI and histology.

## 2. Methods

We employ the well established registration approach based on mutual information (MI) and thin-plate splines (TPS). MI measures the similarity between two scans, while TPS is used to implement the geometric transform. Control points are used to control the degrees of freedom (DOF) in TPS. Registration with many control points can model complex deformation. The process of registration can be formulated as maximizing MI under a hypothetical geometric transform implemented by TPS,

$$\hat{T} = \arg\max_{T} MI(A(\bullet), B(T(\bullet))), \quad \hat{T}; \text{estimate of the transform}.$$

Applying the above registration directly to register histology slides and *ex vivo* MRI is difficult. Such direct registration is a difficult 2D (slice) to 3D (volume) registration, as there may be many portion of the 3D volume that might look similar to the slice in question. Information content in one slice is often not enough to correctly register onto a volume. Histology slides are typically taken at much higher resolution than *ex vivo* MRI, which leads to resolution mismatch. Registration is best accomplished when both scans are of similar resolution. Our approach is to break this difficult direct registration into two easier registration tasks. We acquire digital photographs of prostate specimen (referred to as block face photos) as it is sectioned for histological examination. First, we perform 2D to 2D registrations between histology slides and block face photos using 9 control points. This registration is quite feasible as histology slides are prepared from the sections and many common features can be found in both histology slides and block face photos. Second, we stack block face photos to form a volume. Successive block face photos are registered in rigid (rotate-translate) fashion and then stacked. We place rigidly registered photos in the same spacing used to section the prostate (2.2 mm) and insert zero valued slices in between so that individual slice thickness is thinner. We use 4 zero slices between non-zero slices (photo slices), thus slice thickness is 2.2/5 = 0.44 mm. If we don't insert zero valued slices, it will make regular photo slices (non-zero slices) too thick, which is far from the reality, since histology slides are very thin (0.4 μm). Inserting many zero valued slices will make non-zero slices thinner but it will lead to partial volume effects (resolution mismatch) in the next registration step. Third, we perform a 3D to 3D registration between stacked block face photos and *ex vivo* MRI using 18 control points. If one photo slice is to be incorrectly registered in the *ex vivo* MR then the adjacent slices below and above will discourage that slice in question to be registered onto a wrong position. Basically, adjacent slices will lead the slice in question to achieve better registration. Registration is best achieved when both volumes have similar voxel dimension, thus we cannot make stacked photo volume have far smaller thickness than the thickness of *ex vivo* MRI (0.23 mm). Finally, we combine registration results from three tasks and achieve registration between histology slides and *ex vivo* MRI. The stacked block face photo volume is the key bridge that connects between histology and *ex vivo* MRI. Below is the block diagram of our approach.

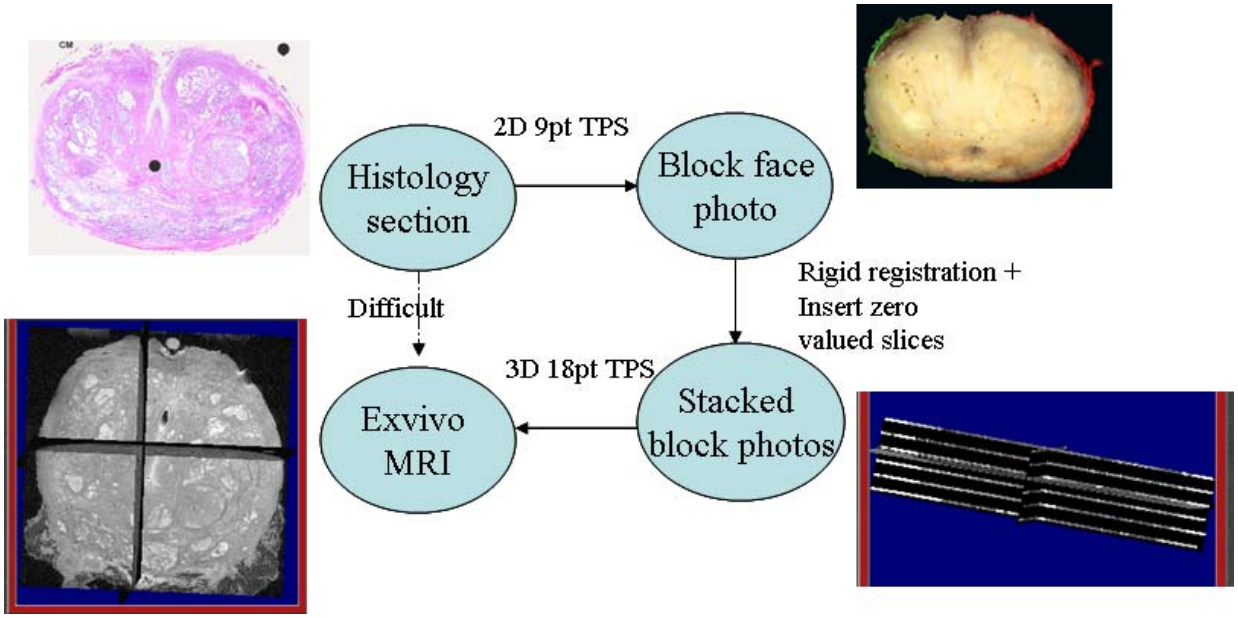

Figure 1. Block diagram of our registration approach. NEEDS TO BE VIEWED IN COLOR.

**3. Results**

Prostate undergoes a formalin fixation after surgery and then histology sections are obtained by whole prostate step section method with 0.4 μm thickness at 2.2 mm spacing. There are 18 histology slides and corresponding 18 block face photos. Block face photos are taken as the sectioning occurs using a regular digital camera. *Ex vivo* MRI is a $T_2$ weighed scan of grid $256^3$ and voxel size $0.23^3$ $mm^3$ acquired from a 7T scanner imaged for roughly 7 hours. Here we show registration results of histology slides and *ex vivo* MRI for one patient. Only histology slice 4 is shown here due to limited space.

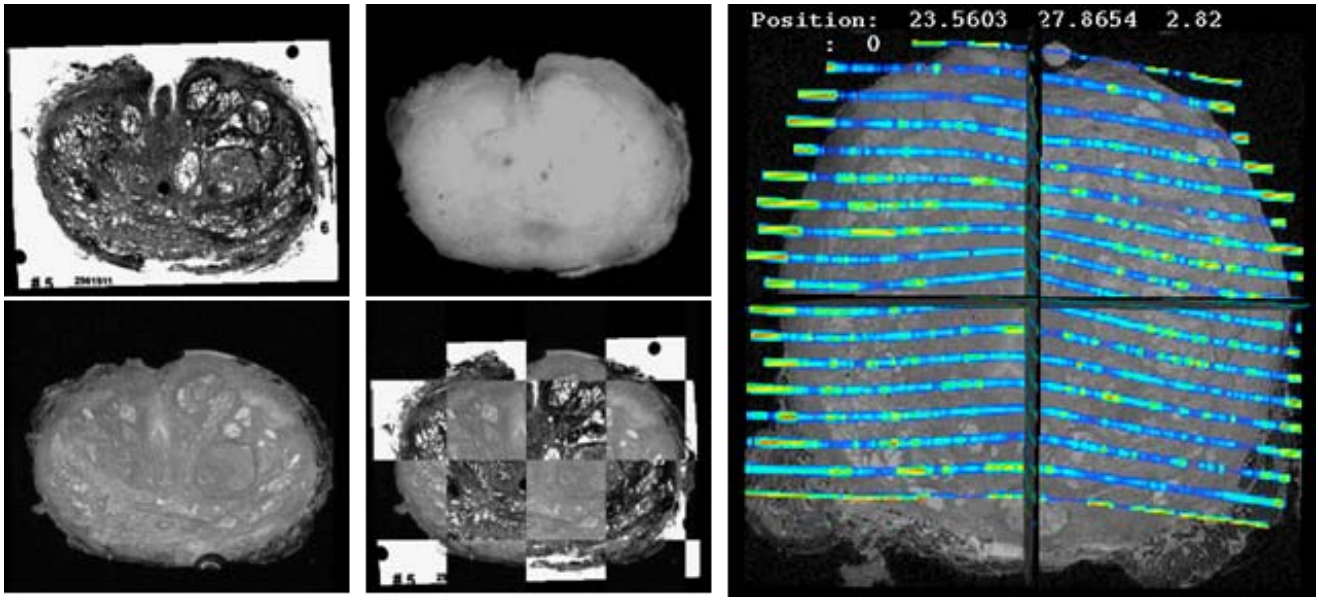

Figure 2. Registration result of one histology slice (slice4) and *ex vivo* MR via intermediate stacked block face photo. NEEDS TO BE VIEWED IN COLOR. Top left is histology, top middle is block face photo, bottom left is *ex vivo* MRI, and bottom middle is fused histology with MRI using alternating subblocks. Above four pictures are all in the same space so that they can be compared quantitatively. Note that all the spatial features line up correctly. Rightmost figure is the volumetric registration between stacked block face photo (colored bright, all 18 slices) and *ex vivo* MRI (grayscale) to visualize the registered slice profile. Note that some of slices are slightly warped to get correctly registered onto *ex vivo* MRI. This warping shows that our registration approach is capable of modeling the non-linear deformation that occurs during the sectioning process.

**APPENDIX 3:**

Curriculum Vitae of Principal Investigator

Attached on the next 5 pages

## BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

| NAME | POSITION TITLE |
|---|---|
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EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

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| Carnegie Mellon University, Pittsburgh, PA | B.S. | 1987-1991 | Electrical Engineering |
| University of Pittsburgh, Pittsburgh, PA | M.D. | 1992-1996 | Medicine |
| University of Hawaii Residency Program, Honolulu, HI | | 1996-1999 | Internal Medicine |
| University of Washington, Seattle, WA | | 2000-2002 | Nuclear Medicine/PET |

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| 1988-1991 | Operations Research Programmer, PPG Industries & Department of Economics, Carnegie Mellon University, Pittsburgh, PA |
| 1991-1992 | Research Assistant, Neurobehavioral Studies Program, Western Psychiatric Institute and Clinic, Pittsburgh, PA |
| 1996-1999 | Internship and Residency, University of Hawaii Internal Medicine Residency Program, Honolulu, HI |
| 1999-2000 | Physician, Internal Medicine and Urgent Care, Waianae Coast Comprehensive Health Center, Waianae, HI |
| 1999-present | Medical Staff, The Queen's Medical Center, Honolulu, HI |
| 1999-2000 | Medical Staff, Saint Francis Medical Center, Ewa Beach, HI |
| 2000-2002 | Fellow, Nuclear Medicine and PET, University of Washington, Seattle, WA |
| 2001-2002 | Staff Physician, Emergency Department, Department of Veterans Affairs-Puget Sound Health Care System, Seattle, WA |
| 2001-2003 | Medical Officer, Seattle Division- Department of Veterans Affairs, Puget Sound Health Care System, Seattle, WA |
| 2002-2004 | Research Fellow, The Queen's Medical Center, Honolulu, HI |
| 2004-2005 | Research Associate, The Queen's Medical Center, Honolulu, HI |
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| 2005-present | Research Director, Hamamatsu/Queen's PET Imaging Center, Honolulu, HI |

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| 2003-2005 | Member, Brain Imaging Council, Society of Nuclear Medicine, Term 2003-2005. |
| 2004-present | Assistant Professor, Department of Medicine, University of Hawaii John A. Burns School of Medicine, Honolulu, HI |
| 2004-present | Clinical Assistant Professor, Department of Geriatric Medicine, University of Hawaii John A. Burns School of Medicine, Honolulu, HI |
| 2004-present | Member, Cancer Committee, The Queen's Medical Center, Honolulu, HI |
| 2005-present | Member, University of Hawaii, Cooperative Institutional Review Board (IRB) |
| 2005-present | Associate Member, University of Hawaii Cancer Research Center Clinical Sciences Program |

## CERTIFICATION

1999 American Board of Internal Medicine
2002 American Board of Nuclear Medicine
2004 Certification Board of Nuclear Cardiology

## HONORS AND AWARDS

Research Scholarship in Neuropsychiatry, University of Pittsburgh Medical Center, 1991

Medical Student Research Excellence Award, University of Pittsburgh School of Medicine, 1993.

Scientific Presentation Award, Annual Straehley Symposium, Kaiser Foundation, November 1997.

Invited Reader, Japan-US Joint Film Reading Conference. 41st Annual Meeting of the Japanese Society of Nuclear Medicine, October 2001.

Residency Award, Asa Seeds Award (Nuclear Medicine), University of Washington, 2002

## PROFESSIONAL MEMBERSHIPS

2005-present Society for Molecular Imaging
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1996-present American College of Physicians
1996-present American Medical Association
1996-present Hawaii Medical Association
1994-1996 Society of Neuroscience
1991-1996 IEEE (Institute for Electrical and Electronics Engineers)

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Kwee SA, Minoshima S. *Japan-US Joint Film Reading Conference. 41st Annual Meeting of the Japanese Society of Nuclear Medicine, October 2001.*

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**FEDERAL GRANTS**

PC040130 7/2004 – 7/2007
Cancer Localization in the Prostate with F-18 Fluorocholine Positron Emission Tomography
Congressionally Directed Medical Research Programs
Role: Principal Investigator

R41CA110121 9/2004 – 3/2006
Positron Emission Tomography Guided Prostate Biopsy
National Institutes of Health, National Cancer Institute
Role:Co-Investigator

**PAST RESEARCH SUPPORT**

The Holy Land Foundation, The James and Sally Zukerkorn Foundation, The Queen Emma Research Foundation, Hamamatsu Photonics KK, Philips Medical Imaging.